FINAL REPORT

Whitebark pine (*Pinus albicaulis*) growth and defense characteristics in disturbance-prone high elevation montane ecosystems of the northern Rocky Mountains

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List of Abbreviations / Acronyms

BAI   Basal Area Increment
CSKT   Confederated Salish-Kootenai Tribes
DBH   Diameter at Breast Height
RWI   Ring Width Index

Keywords

whitebark pine, growth, defense, resin ducts, resin chemistry, mountain pine beetle, disturbance, mortality

Acknowledgements

Abstract

Recent, large-scale tree mortality in the western U.S., resulting from changes in climate, pathogens, insect activity, and forest management practices has led to concerns for many ecologically and culturally important species. Given the ecological implications of large-scale mortality events, there is growing interest in better understanding physiological strategies employed by trees in response to these shifting disturbance patterns. Within conifers, resin-based defenses have long been recognized as a primary defense mechanism against a variety of insects and pathogens. Whitebark pine is a critical high-elevation species that provides a host of ecological services within subalpine and alpine ecosystems. As whitebark pine has evolved with many biotic and abiotic disturbances, individual trees generally exhibit well-developed resin-based defenses with extensive networks of constitutive resin duct structures, making it a good candidate species to study growth and defense relationships. In this study we compared growth and resin duct structures between pairs of whitebark pine that persisted through 21st century mountain pine beetle outbreaks (hereafter "live trees") and trees that died during these disturbance events (hereafter "dead trees"). We also compared constitutive resin chemistry, growth, and resin ducts for a subset of whitebark and lodgepole pine growing in proximity within a disturbance-prone, mixed-conifer forest in northwestern Montana. We found that live and dead trees exhibited notably different growth and defense characteristics. Live whitebark pine produced 56% larger resin ducts with a 57% greater annual investment in resin-based defenses than whitebark pine trees that died. In contrast, whitebark pine that died exhibited 22% greater growth over the full time series than their live counterparts and also produced 20% more resin duct structures on average, prior to mortality. Resin duct size, duct area, and relative duct area were the most important variables influencing whitebark pine survivorship. Trees producing larger ducts with a greater overall percentage of annual carbon investment were far more likely to survive disturbance events. Regarding constitutive resin chemistry and growth / defense relationships we found four important findings: 1) We did not find evidence of a tradeoff between tree growth and tree defenses (resin duct morphology and constitutive resin chemistry). This suggests that trees growing under favorable field conditions can experience high growth rates and be well defended. 2) Resin ducts and constitutive mono- and sesqui- terpenes were not related in lodgepole pine while duct production, size, and area were positively related to constitutive terpenes in whitebark pine. This suggests that trees can invest differentially in primary and secondary metabolites, presenting beetles with many permutations of complex resin-based defenses, particularly from its historical host. 3) Differences in whitebark and lodgepole pine constitutive resin chemistry relate to bark beetle behavioral recognition, semiochemical communication, and attack success. These chemical differences are consistent with field patterns by which mountain pine beetles are more likely to enter lodgepole pine but are more likely to succeed through initiation of mass attack in whitebark pine. 4) Overstory competition, especially by Engelmann spruce, can influence constitutive terpenes. With increased warming, tree species may migrate through zones of transition where there is increased susceptibility to mountain pine beetle. This warrants further investigation.

Objectives

The primary objective of this research was to investigate growth and defense characteristics (resin duct anatomy and resin chemistry) of whitebark pine in stands that have experienced recent mountain pine beetle outbreaks and whitepine blister rust exposure. The following questions motivated this research:

1) Are there physiological differences between whitebark pine that have persisted through recent stand-level disturbances (bark beetles and blister rust) and those that have died?

2) What growth and defense characteristic(s) are most important for residual live trees?

3) Are there correlations among resin chemistry, tree growth, and resin duct anatomy?

4) Is there a difference in resin chemistry and growth and defense relationships between whitebark and lodgepole pine?

5) Does the abundance and diversity of overstory and understory competition influence resin chemistry for either whitebark or lodgepole pine?

The lack of empirical studies evaluating growth and defense relationships for high elevation pines that experience multiple, complex disturbance stressors, highlights a need to better understand growth and defense characteristics across a range of biophysical gradients. Understanding resin defense systems is of particular importance in this regard as these advanced biochemical structures represent the primary defense mechanism of whitebark pine to biotic disturbance. Evaluating relationships between resin duct structures and oleoresin production and disturbance can provide valuable insight into overall defensibility of these trees to stressors that are projected to increasingly impact this important cultural and ecological species.

Background

Recent, large-scale tree mortality in the western U.S., resulting from changes in climate, pathogens, insect activity, and forest management practices has led to concerns for many ecologically and culturally important species (Schwandt et al. 2010, Tomback and Achuff 2010, Roy et al. 2014). Given the ecological implications of large-scale mortality events, there is growing interest in better understanding physiological strategies employed by trees in response to these shifting disturbance patterns. However, tree physiology varies considerably within and across species and disturbance regimes fluctuate substantially across both time and space. This complicates our ability to make generalized assumptions and hinders development of effective management solutions that account for complex species-specific responses to biotic and abiotic processes.

Within conifers, resin-based defenses have long been recognized as a primary defense mechanism against a variety of insects and pathogens. Resinous compounds are produced, stored, and mobilized within a network of resin duct structures that are connected vertically and horizontally throughout the tree, facilitating rapid mobilization of resins to sites of injury or infection (Hood and Sala 2015, Celedon and Bohlmann 2019). Resin ducts are embedded within annual growth rings (secondary xylem tissues) and can be measured over time to help quantify how trees allocate carbon between growth and constitutive defenses.

Several studies have found relationships between resin duct characteristics (e.g., number of ducts produced, duct size, area, density) and increased probability of survival during bark beetle outbreaks (Kane and Kolb 2010, Ferrenberg et al. 2014, Hood et al. 2015, Hood et al. 2016), drought (Gaylord et al. 2007, Gaylord et al. 2013, Gaylord et al. 2015), and wildfire (Hood et al. 2015, Hood et al. 2016, Slack et al. 2016, Sparks et al. 2017). However, duct anatomy is highly variable within and across species, complicating our understanding of how these defensive features function over diverse biogeographic gradients (Ferrenberg et al. 2014, Hood and Sala 2015, Kane et al. 2017, Kichas et al. 2020).

In addition to resin duct anatomy, an important component influencing tree survival in the presence of insect disturbance agents is the chemical composition of the resin itself (Duhl et al. 2013, Raffa 2014, Keefover-Ring et al. 2016). Oleoresin contains complex mixtures of terpenoids (mono-, sesqui-, and di- terpenes) that have demonstrated linkages to insect behavior and attack success (Phillips and Croteau 1999, Raffa et al. 2013, Raffa 2014, Raffa et al. 2017). While many species of conifers produce similar chemicals (e.g., α-pinene, limonene, β-myrcene), the relative composition and concentration of these compounds vary considerably across taxa as well as with ontological development and local environment (Smith 2000, Moreira et al. 2014, Raffa 2014, Moreira et al. 2015, Moreira et al. 2016). This variability leads to diverse and largely unknown interactions with insects and pathogens that can facilitate significant change in forest composition and structure during periods of increased disturbance.

Whitebark pine is a critical high-elevation species that provides a host of ecological services within subalpine and alpine ecosystems (Logan et al. 2010, Tomback et al. 2016a, Tomback et al. 2016b, Wagner et al. 2018). As whitebark pine has evolved with many biotic and abiotic disturbances, individual trees generally exhibit well-developed resin-based defenses with extensive networks of constitutive resin duct structures, making it a good candidate species to study growth and defense relationships. However, there is growing concern that whitebark pine may be largely extirpated from its current habitat over the next century due to cumulative impacts of climate change, insect-related mortality, changing fire regimes, increased competition from shade-tolerant species, and the invasive exotic pathogen causing white pine blister rust (*Cronartium ribicola*). These stressors, primarily blister rust, have already decimated populations, by as much as 90% in some areas of the northern Rockies (Keane and Arno 1993, Kendall and Keane 2000, Shanahan et al. 2016, Amberson et al. 2018). In December 2020, the U.S. Fish and Wildlife Service formally proposed whitebark pine as a "threatened" species under the Endangered Species Act, which if approved will require careful and adaptive management to sustain and promote remaining whitebark pine populations.

Some work has investigated the physical and chemical defenses of whitebark pine trees in the Greater Yellowstone Ecosystem (Raffa et al. 2013, Bentz et al. 2015, Raffa et al. 2017, Mason et al. 2019). However, these studies are representative of only a portion of the broader range of whitebark pine, precluding inference to other forest types and establishing a need to conduct similar types of research across a broader range of environments.

In this study we compared growth and resin duct structures between pairs of whitebark pine that persisted through 21st century mountain pine beetle outbreaks (hereafter "live trees") and trees that died during these disturbance events (hereafter "dead trees"). We also compared growth, resin ducts and resin chemistry for a subset of lodgepole and whitebark pine growing in proximity within a disturbance-prone, mixed-conifer forest in northwestern Montana.

Materials and Methods

Study Sites

Data for this study were collected on two high elevation whitebark pine sites on the Flathead Indian Reservation during the summer of 2016 as part of a larger fire history reconstruction for the Confederated Salish and Kootenai Tribes (CSKT; **Figure 1**). We selected sites where whitebark pine was an important component of the canopy (> 15 % canopy cover). Canopy species at both sites consist of lodgepole pine (*P. contorta* var *latifolia*) and whitebark pine, with smaller components of subalpine fir (*Abies lasiocarpa*) and Engelmann spruce (*Picea engelmannii*).

The 3LK site is located in the Reservation Divide Mountains between the Flathead Indian Reservation and Missoula, Montana. Whitebark pine comprised 15% of all sampled individuals (225 out of 1,507 trees). The majority of whitebark pine mortality at this location was due to cumulative impacts from mountain pine beetle and white pine blister rust, which was introduced to this region of the Northern Rocky Mountains circa 1950 (Samman et al. 2003, Geils et al. 2010). Of the 225 sampled whitebark pine trees, 73% were dead, with the majority of dead trees (88%) showing evidence of beetle activity (*J*-shaped galleries along the tree stem and / or presence of blue-stain fungus (*Grosmannia clavigera*), which is introduced by bark beetles during colonization.

The BLD site is located approximately 74 kilometers away in the Mission Mountains on the Eastern side of Flathead Lake. Whitebark pine was more prominent at this site, comprising 36% of all sampled individuals (476 out of 1,320 trees). Of these, 14% were live and 86% were dead, the majority of which (71%) had evidence of bark beetle activity.

Both sites are characterized by mixed- and lethal-severity fire regimes and had recent histories of bark beetle activity in the 1930s, 1960s–1980s and 2002–2009 (Arno and Hoff 1989, Kipfmueller and Swetnam 2002, Buotte et al. 2017, van de Gevel et al. 2017).
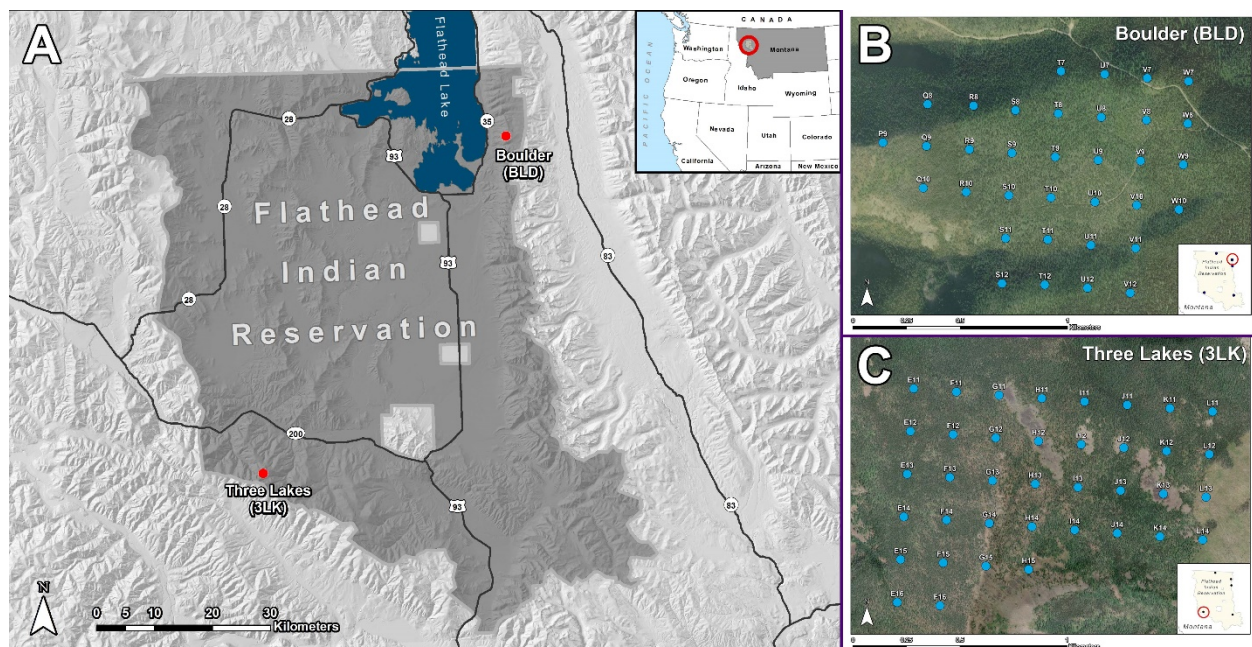
Figure 1. Map of the Flathead Indian Reservation and two sites used for this research (A). The Boulder site sits on the western slopes of the Mission Mountains (B) while the Three Lakes Peak site is located in the Reservation Divide Mountains (C).

Data Collection

As part of the CSKT fire-history reconstruction effort, a 200 m grid was overlain across the study area using a combination of remote sensing and field surveying for optimal placement (**Figure 1**). This gridded sampling design effectively captured much of the topographic and vegetative variability across each site, providing for a more accurate assessment of disturbance legacies and microsite differences in forest structure and stand composition. Within the grid, individual plots (hereafter "macroplots") were located 200 m apart along cardinal directions, with a total of 34 plots for the BLD site, covering approximately 88 ha (**Figure 1B**), and 38 plots for the 3LK site, covering approximately 100 ha (**Figure 1C**).

Each macroplot was spatially referenced and plot centers were permanently marked and tagged. From the macroplot center, four 10 m wide, 100 m long belt transects were established in each cardinal direction (north, south, east, and west; **Figure 2A**). The closest ten trees within each 100 m transect were sampled (to a maximum distance of 100 m) with a diameter threshold of 15 cm diameter at breast height (DBH) (Heyerdahl et al. 2014). Data collected for each tree at each macroplot included species, condition (live or dead), size (DBH), canopy base height, crown class (dominant, codominant, intermediate, or suppressed), tree height, and evidence of fire. We collected increment cores from 40 sample trees per macroplot near the base of each tree (at or below 15 cm from the root collar) using Haglöf 4.3 mm diameter increment borers. A 25 m^2 circular microplot was also established around plot center to estimate percent cover of understory species (**Figure 2A**).

Live / Dead Tree Pairing

To assess the influence of disturbance on whitebark pine physiology (growth and defense characteristics) live trees and corresponding dead trees (hereafter "pairs") were identified from the macroplot data. Suitable pairs were identified based on distance ($\leq$ 20 m apart) and size ($\pm$ 3 cm DBH) to control for potential microsite differences (Kane and Kolb 2010, Hood and Sala 2015). As a result of these pairing criteria, only a subset of the macroplots contained suitable pairs for this analysis. Overall, 30 pairs were identified at the 3LK site across 13 macroplots and 42 pairs were identified at the BLD site across 13 macroplots for a total of 144 sampled trees (72 live and 72 dead).

Phloem Tissue Collection

At the BLD site, we identified 30 live whitebark and lodgepole pines from the macroplot data for phloem collection so that we could investigate constitutive resin chemistry. To control for microsite variability and visible differences in ontogeny we paired trees based on size ($\pm$ 3 cm DBH) and distance ($\leq$ 15 m apart). In June 2018, individual trees were located in the field. To assess the potential influence of overstory competition on resin chemistry, we recorded species and DBH information for all live and dead trees ($\geq$ 1 cm DBH) within five meters of each subject and for all trees ($\geq$ 5 cm DBH) to a distance of ten meters (**Figure 2B**). For understory competition, we identified species and estimated percent cover within a 5.6 m diameter microplot around each subject tree. We collected phloem tissue of approximately 1.5 cm width

and 5 cm length for all 60 individuals. Tissue samples were immediately placed in glass vials on dry ice and were transported to Montana State University for storage in a –80° F freezer within 24 hours of collection.
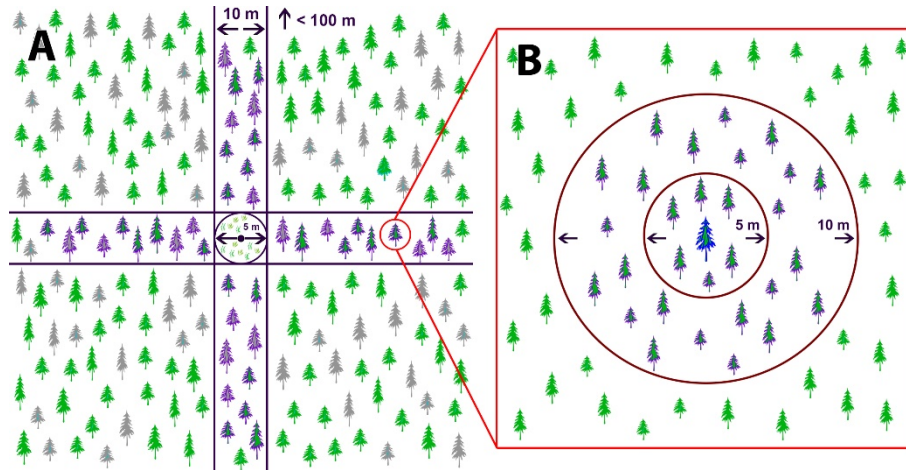
Figure 2. Conceptual diagram of sampling protocol. (A) from plot center, four 10 m wide belt transects were extended in each cardinal direction and the closest 10 trees (> 15 cm DBH) in each transect were sampled (to a maximum distance of 100 m). A 5.6 m diameter plot was established around plot center to measure herbaceous vegetation. (B) pairs of whitebark pine and lodgepole pine trees were sampled in 2018 for resin chemistry and competition. Species and DBH information were obtained for all trees > 1 cm DBH within 5 m while all trees > 5 cm DBH were recorded to a distance of 10 m.

Data Analysis

Live / Dead Tree Pairs

All analyses were conducted using R (v. 3.5.0) (R Development Core Team 2008). We calculated growth and defense metrics annually (for all overlapping years between live and dead trees), as well as in 20-, 10-, and 5-year periods corresponding to the 20-, 10-, and 5-year windows prior to mortality (**Figure 3**). For growth measures, we utilized ring area (ring width * core diameter) as well as a standardized, unitless ring width index (RWI). The RWI was developed through interactive detrending in the *dplR* package (version 1.64) (Bunn 2008) in R (version 3.31) (R Development Core Team 2008).

We used Wilcoxon's paired *t*-tests to assess differences in growth and defense between pairs of live and dead whitebark pine at each site and applied a Bonferroni correction factor ($\alpha = 0.05/4 = 0.0125$) for all growth periods to account for multiple comparisons across similar growth periods (e.g. mean growth for total, 20-, 10-, and 5-year time periods). We utilized a combination of multivariate analyses of variance, correlation analyses and regression analyses to examine relationships between annual radial growth (ring area and growth index) and defense metrics (resin duct size, production, area, density and relative area).

We developed a whitebark pine probability of mortality model using logistic regression. Live and dead whitebark pine were pooled across both sites yielding 144 samples. Mortality models were evaluated using an information theoretic approach, whereby a variety of candidate models were initially developed from the suite of growth and defense metrics and compared using Akaike's Information Criterion (AIC). We constructed ten models utilizing various combinations

of growth and defense metrics following *a priori* assumptions (e.g. that a combination of resin duct size and duct area would collectively influence resin flow and may therefore be important variables influencing survivorship). Model parameters were limited to five variables or less and growth variables for overlapping periods were excluded within any given model to avoid issues of multicollinearity (e.g. 5-year ring area and 10-year ring area). To assess which growth and defense variables were most important in distinguishing between live and dead trees, we also developed conditional density plots using our full dataset.
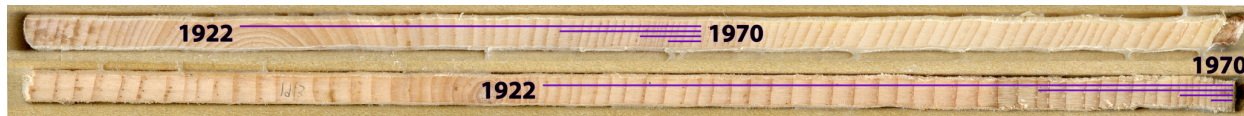

Figure 3. Example of increment cores from a pair of live (top) and dead (bottom) whitebark pine. Trees were paired based on distance (≤ 20 m) and size (± 3 cm DBH). Growth and defense measures were compared across all overlapping years, as well as 20-, 10-, and 5-year periods (series of progressively shorter purple lines in the figure).

Phloem Chemistry

We used a combination of Wilcoxson's paired *t*-tests, linear regression, correlation, and multivariate analyses of variance to investigate relationships in growth and defense anatomy, as well as constitutive resin chemistry, across lodgepole and whitebark pine pairs. Growth and defense metrics were measured over the full record (1914–2018) as well as over the most recent 20-years (1998–2018) prior to sampling. We applied a Bonferroni correction factor ($\alpha = 0.05/2 = 0.025$) for all growth periods. From the raw tree ring width data we used the *dplR* package (v. 1.7.1) (Bunn 2008) to calculate cross-sectional basal area increments (BAI; mm^2 $year^{-1}$) as a measure of volumetric growth over time (Biondi and Qeadan 2008).

For mono- and sesqui- terpenes, we standardized concentrations by mass of the phloem sample (g) to calculate absolute concentration (µg / g phloem) for each compound. We then summed the concentrations for all identified compounds to obtain total concentration (41 identified monoterpenes and 39 identified sesquiterpenes). For individual terpenes, we calculated the relative concentration of each compound as percent of the total concentration. A one-way Welch's ANOVA was used to test for a species effect on total mono- and sesqui- terpenes, followed by a Tukey's honest significant difference test (HSD) *post-hoc*. Due to non-normally distributed data, mono- and sesqui- terpene amounts were either square root or log-transformed for these analyses. We also used non-metric multidimensional scaling (NMDS) to explore mono- and sesqui- terpene dissimilarity between the two species (Kenkel and Orlóci 1986, Dixon 2003). Dissimilarity for the NMDS ordination was calculated using the *betadisper* function in the *vegan* package (v. 2.5–6) in R (Dixon 2003).

To assess the role of competition we summed basal area (m^2) for each species of competitor and for all trees sampled around each focal tree (**Figure 2B**). From these data we calculated stand density index (SDI) (Reineke 1933) for each tree and developed generalized linear models to assess whether local competition and/or canopy position, influence constitutive levels of mono- and sesqui- terpenes and/or growth and resin duct anatomy. All models were compared using Akaike information criterion (AIC) (Burnham et al. 2010).

Results

Live / Dead Tree Pairs

Principal components analysis showed a clear distinction in past growth and defense characteristics between live and dead whitebark pine (**Table 1, Figure 4**). Resin duct size, duct area, duct density and relative duct area were the most important variables contributing to the first principal component (PC1), cumulatively describing 45.8% of variability within the data. Growth (ring area) and resin duct production had the greatest contribution to PC2, cumulatively describing an additional 28.9% of variability within the data (74.7% total variability).

Table 1. Age of live and dead whitebark pine at study locations on the Flathead Indian Reservation.

	3LK		BLD	
	Live	*Dead*	*Live*	*Dead*
Age (Range)	72 – 118	41 – 100	94 – 113	44 – 101
Age (Average)	98 ± 1.9	75 ± 3	105 ± 0.9	77 ± 1.9

Note: Majority of trees died during late 1960s–1990s and exhibited signs of beetle activity (J-shaped galleries on tree bole and blue-staining in increment cores). Overall trends in growth and defense metrics were similar at each site, so the full suite of data was merged for analysis (n = 144 trees).
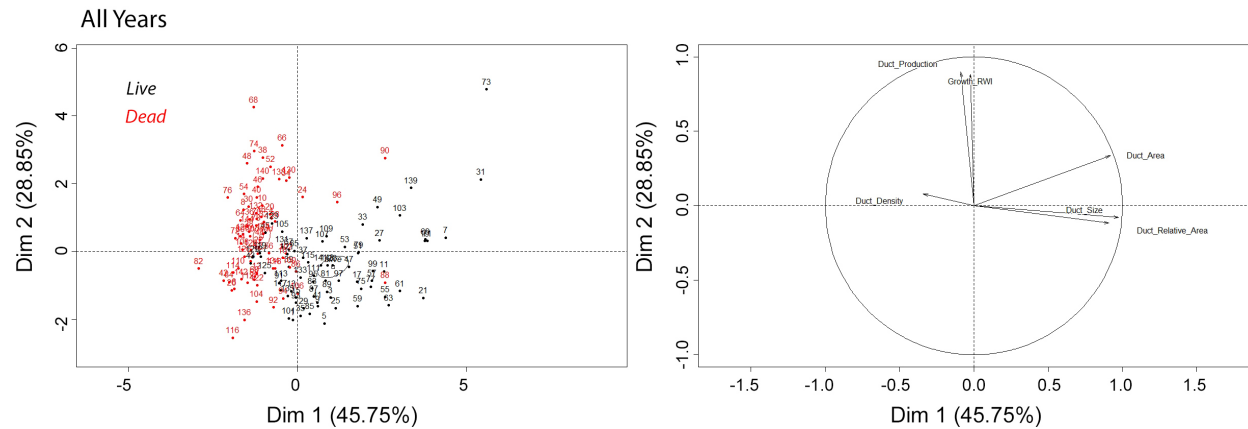
Figure 4. Principal components analysis comparing growth and resin duct characteristics (production, size, area, density, relative area) for live and dead whitebark pine (144 samples, 72 live, 72 dead). Ellipses are 95th percentile confidence intervals for each grouping (live - black; dead - red). Variables factor map (right panel) illustrates the relative contribution of growth and defense metrics to the ordination.

Whitebark pine that had died had grown 22% faster (*p*-value < 0.0001) than living trees during the full record, although this pattern was driven by earlier years (1911–1975). In the 20-years preceding mortality, growth in whitebark that died declined by 26% relative to live trees, especially post-1975 (**Figure 5A**). Dead whitebark pine also produced more resin ducts compared to live trees over the full record (20% greater production; *p*-value < 0.0001). This relationship declined (by 10%) in the 20-years preceding mortality, with the greatest difference occurring during a small interval from around 1990–2000 (**Figure 5B**). However, despite producing more resin duct structures on average, the resin ducts were smaller for dead whitebark pine (56% smaller on average) compared to live trees (*p*-value < 0.0001). Similar to growth, duct

size showed an increasing trend post-1975, where duct size in live trees continued to increase relative to dead trees (**Figure 5C**).

Resin duct area was also greater in live trees across the full record (48% increase; *p*-value < 0.0001) and duct area showed a similar post-1975 trend, with increasing area in live whitebark pines relative to dead trees (**Figure 5D**). In contrast, resin duct density was greater in dead trees (18% greater; *p*-value = 0.0149) and post-1975, duct density continued to increase in dead whitebark pine throughout the remainder of the record (**Figure 5E**). Relative duct area (% of annual ring occupied by resin ducts) was significantly greater in live whitebark pines (57% increase; *p*-value < 0.0001). Unlike the other growth and defense metrics, there was no clear temporal trend evident for relative duct area (**Figure 5F**).
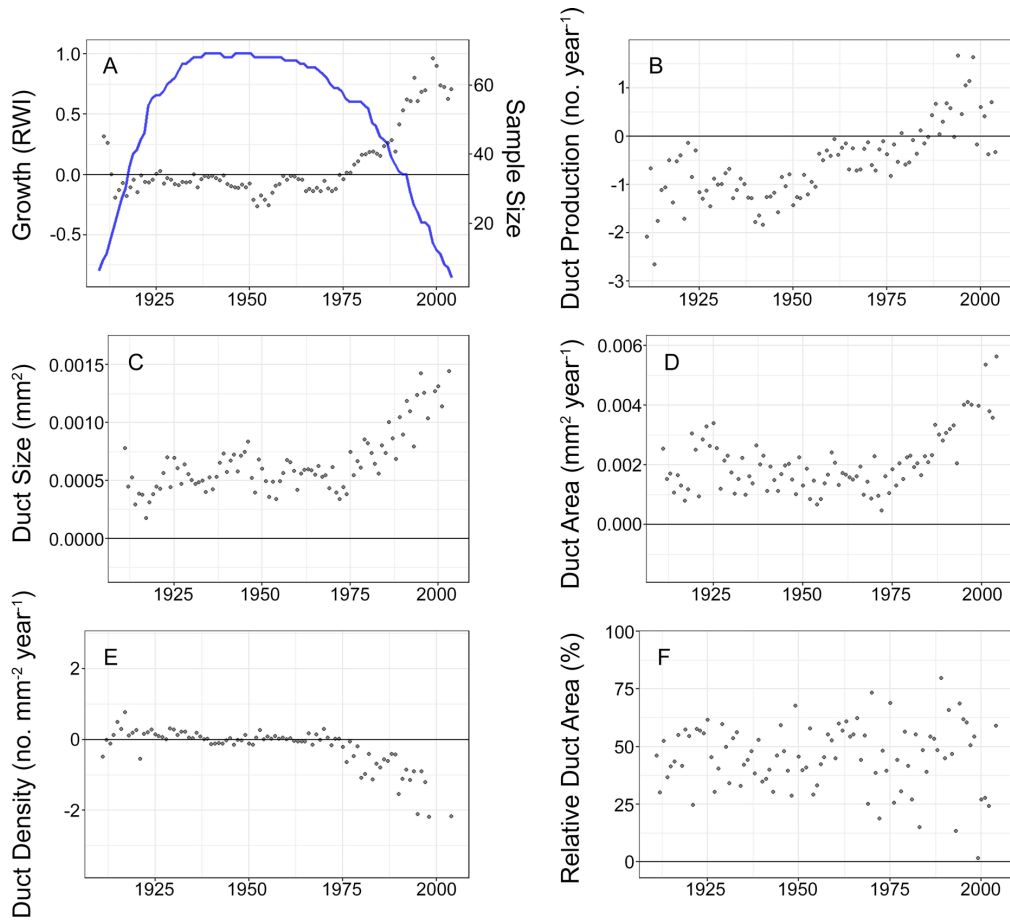
Figure 5. Time series plots showing the difference in growth and defense metrics between live and dead whitebark pine (144 samples, 72 live, 72 dead). Each point represents the difference in growth / defense metrics for a pair of trees (1 live / 1 dead). Points with positive values reflect a greater response for live trees while points with negative values reflect a greater response for trees that ultimately died (formula = [live tree metric] – [dead tree metric]). Blue line in upper-left plot illustrates sample depth across the time series.

The two most significant metrics influencing tree survivorship were resin duct size (mm²), and relative duct area (% of annual ring). Whitebark pine trees that are able to produce larger resin

ducts (> 0.01 mm^2) with greater overall duct area ($> 1\%$ annual ring) had a significantly greater chance of survival (~80%; **Figure 6**).

The best mortality model included resin duct size within the 5-years prior to mortality and relative resin duct area across the full record (**Table 2**). The top five best performing mortality models all included relative duct area (for the full record), highlighting the importance of this metric in differentiating live and dead trees (**Figure 6**). The top performing models also included a combination of resin duct size and duct area across the 5-year and 10-year windows. The only growth variable significant in the top five models was growth (RWI) in the 5-year window (**Table 2**).
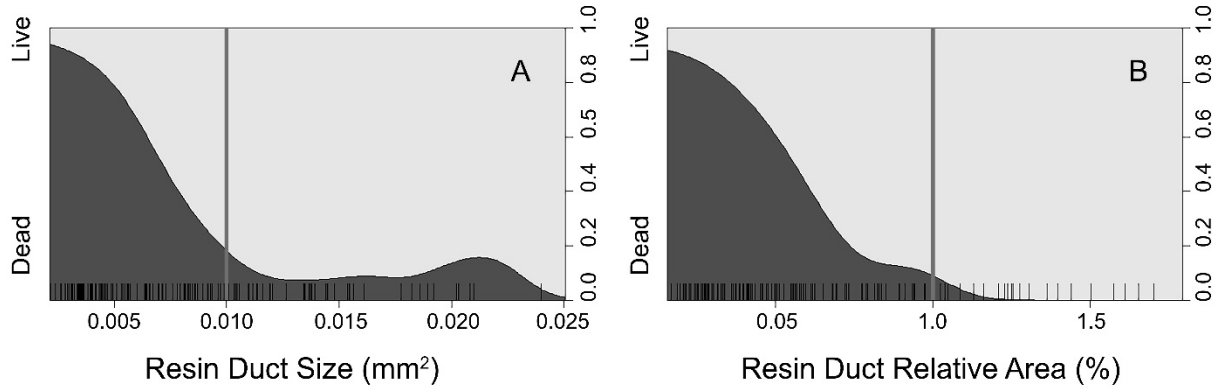
Figure 6. Conditional density plots describing the probability of mortality in relation to principle resin duct metrics (A) resin duct size; mm^2 and (B) relative resin duct area; % annual ring for 144 whitebark pines (72 live and 72 dead). Light shading reflects live trees while dark shading reflects dead trees. Vertical black bars along the x-axis represents the distribution of samples.

Table 2. Generalized linear models predicting survival of whitebark pine during disturbance outbreaks. Log likelihood reflects the probability of the data given the model, while K represents the relative number of sample parameters for each model. Top five models are shown out of ten models developed.

Model	AIC	ΔAIC	LogL	K
5year_Size + Total_Relative_Area	88.2	0	–41.1	2
5year_Size + Total_Relative_Area + 5year_Area	89.7	1.4	–40.8	3
10year_Size + Total_Relative_Area + 5year_Area + 5year_RWI	95.5	7.3	–42.8	4
10year_Size + Total_Relative_Area	97.1	8.8	–42.5	2
10year_Size + Total_Relative_Area + 5year_Area	98.1	9.9	–45.1	3

Abbreviations: Area (resin duct area); Relative_Area (relative resin duct area); Size (resin duct size); RWI (ring width index); 5year (5-year window prior to mortality); 10year (10-year window); Total (all overlapping years).

Relationship between Constitutive Resin Chemistry and Tree Ring Anatomy

Whitebark and lodgepole pine at our study site were similar in terms of size (diameter and height), age, and relative growth (**Table 3**). In general, tree size, tree growth, and resin duct properties correlated poorly with constitutive mono- and sesqui- terpenes in both species. However, across the full time-series (1914–2018) resin duct area positively correlated with monoterpenes ($r = 0.44$, $p = 0.0152$) and sesquiterpenes ($r = 0.44$, $p = 0.015$) for whitebark pine

only (**Table 4**). This held true for whitebark pine duct area and sesquiterpenes over the 20-years prior to sampling ($r = 0.48$, $p = 0.0079$). Resin duct production correlated positively with monoterpenes in whitebark pine ($r = 0.43$, $p = 0.019$), but showed no relationship in lodgepole pine (**Table 4**). Whitebark pine resin duct size correlated positively with sesquiterpenes, but not monoterpenes, across the full record ($r = 41$, $p = 0.0253$). We did not detect any relationships between constitutive mono- and sesqui- terpenes and resin duct density, relative duct area, growth (BAI), or tree size (DBH) for either species (**Table 4**).

Table 3. Mensuration data for 30 whitebark pine (*P. albicaulis*) and 30 lodgepole pine (*P. contorta* var. *latifolia*) sampled at a high elevation montane site on the Flathead Indian Reservation in northwestern Montana. Mean values are depicted as well as standard error in parentheses.

	Pinus albicaulis	*Pinus contorta*
Age (years)	92.2 (± 2.11)	88.6 (± 1.78)
DBH (cm)	24.2 (± 0.81)	24.5 (± 0.84)
Height (m)	20.2 (± 1.31)	21 (± 1.3)
Live Crown Ratio (%)	73 (± 0.02)	74 (± 0.02)
Aspect (°)	188.5 (± 13.02)	177.1 (± 12.48)
BAI (cm^2 year^{-1})	523.7 (± 50.11)	486.7 (± 42.55)
Duct Production (no. year^{-1})	3.2 (± 0.16)	2.9 (± 0.1)
Duct Size (mm^2)	0.021 (± 0.001)	0.024 (± 0.001)
Duct Area (mm^2 year^{-1})	0.038 (± 0.002)	0.039 (± 0.002)
Duct Density (no. mm^{-2} year^{-1})	0.85 (± 0.1)	0.78 (± 0.08)
Relative Duct Area (% annual ring)	0.92 (± 0.05)	1.03 (± 0.06)

Note: DBH = diameter at breast height; BAI = basal area increment

Table 4. Spearman correlation coefficients of mono- and sesqui- terpene concentrations (µg / g fresh weight) with tree ring growth and resin duct anatomical measurements in whitebark (*P. albicaulis*) and lodgepole pine (*P. contorta* var. *latifolia*). Correlations were conducted on metrics averaged over the full time series (1914–2018).

	Monoterpenes		Sesquiterpenes	
	Pinus albicaulis	*Pinus contorta*	*Pinus albicaulis*	*Pinus contorta*
DBH	0.13	0.19	0.28	0.21
Growth (BAI)	0.18	0.24	0.24	0.1
Duct Production	**0.43**	–0.1	0.23	–0.18
Duct Size	0.26	0.34	**0.41**	0.18
Duct Area	**0.44**	0.28	**0.44**	0.06
Duct Density	0.35	–0.18	–0.06	–0.2
Relative Duct Area	0.33	–0.13	0.003	–0.22

Note: Bold values indicate significant correlations ($p < 0.05$). DBH = diameter at breast height; BAI = basal area increment

Phloem Constitutive Resin Chemistry

Whitebark pine contained 31% greater abundance (µg / g of sample tissue) of constitutive monoterpenes ($p = 0.0193$; **Figure 7A**) and 68% greater abundance of constitutive sesquiterpenes ($p < 0.0001$; **Figure 7B**) compared to lodgepole pine. The diversity of constitutive monoterpenes (calculated as Shannon's *H'*, a measure of within-sample diversity) did not differ significantly between whitebark and lodgepole pine. However, constitutive

sesquiterpene diversity was greater in whitebark pine compared to lodgepole pine ($p < 0.0001$). Overall, we identified 16 monoterpenes that differed considerably across whitebark and lodgepole pine (**Figure 8**) and 28 sesquiterpenes that differed significantly between the two species (**Figure 9**). Importantly among these, we found 6x greater (−)-α-pinene, 8.2x greater (+)-α-pinene and 4.5x greater β-myrcene in whitebark pine. In contrast, lodgepole pine exhibited greater concentrations of α-phellandrene (6.8x higher), β-phellandrene (1.4x higher), (+)-δ-limonene (5.7x higher), and more of the phenolic phenylpropanoid 4-allylanisoleestragole (10.1x higher) than whitebark pine (**Figure 8**).
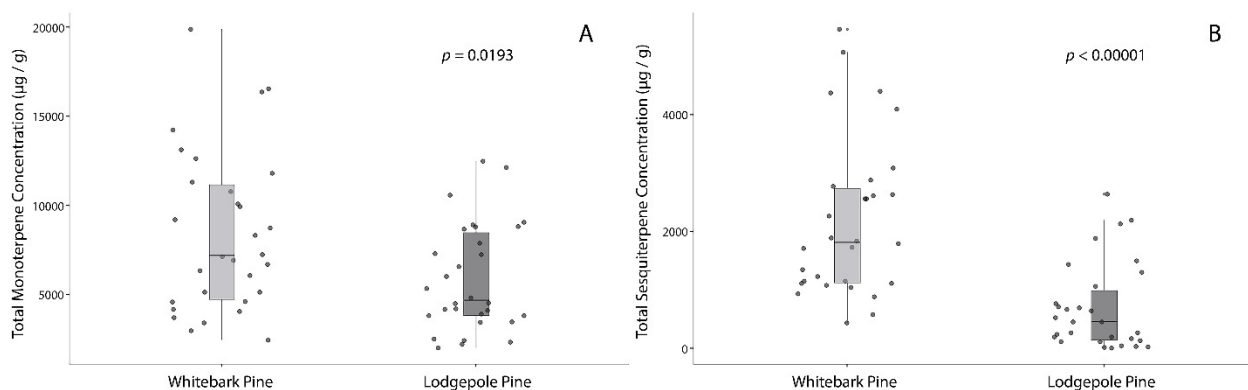
Figure 7. Comparison of total constitutive concentrations (µg / g fresh weight) of monoterpenes (A) and sesquiterpenes (B) across 30 whitebark pine (*Pinus albicaulis*) and 30 lodgepole pine (*Pinus contorta*).
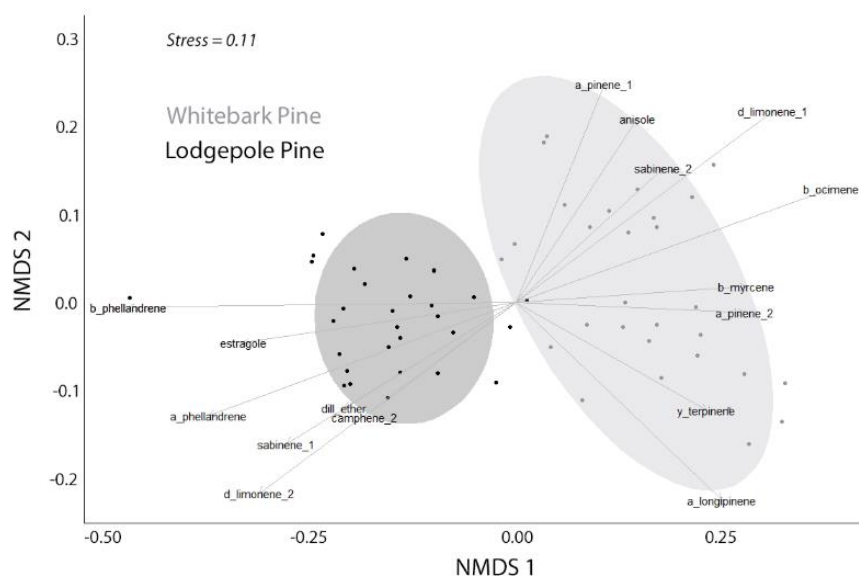
Figure 8. Non-metric multidimensional scaling (NMDS) ordination plot of the first and second dimensions for the monoterpene profiles of 30 whitebark pine and 30 lodgepole pine trees. Ellipses represent a 99% confidence interval for each species. Important individual monoterpenes are overlain with the relative magnitude of the arrows corresponding to their respective importance in the ordination.
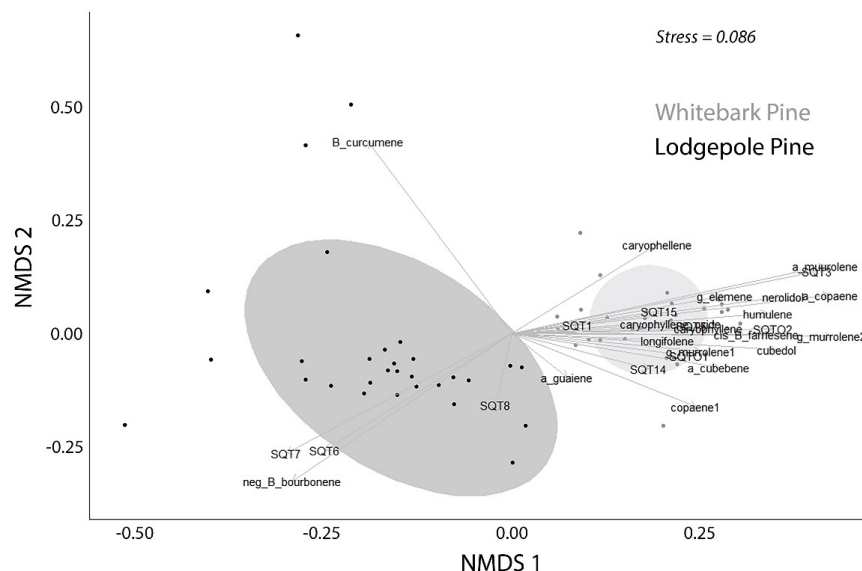
Figure 9. Non-metric multidimensional scaling (NMDS) ordination plot of the first and second dimensions for the sesquiterpene profiles of 30 whitebark pine and 30 lodgepole pine trees. Ellipses represent a 99% confidence interval for each species. Important individual sesquiterpenes are overlain with the relative magnitude of the arrows corresponding to their respective importance in the ordination.

Growth and Defense Characteristics of Whitebark and Lodgepole Pine

Overall, trends in growth and resin duct properties were similar between the two species with a few notable exceptions. Across the full time series (1914–2018) size was the only resin duct metric that differed significantly between the two species, with lodgepole pines producing 10% larger resin ducts on average than whitebark pine ($p = 0.0066$; **Figure 10A**). During the 20-year period prior to sampling whitebark pine produced 22% more resin ducts than lodgepole pine ($p = 0.0207$; **Figure 10B**).
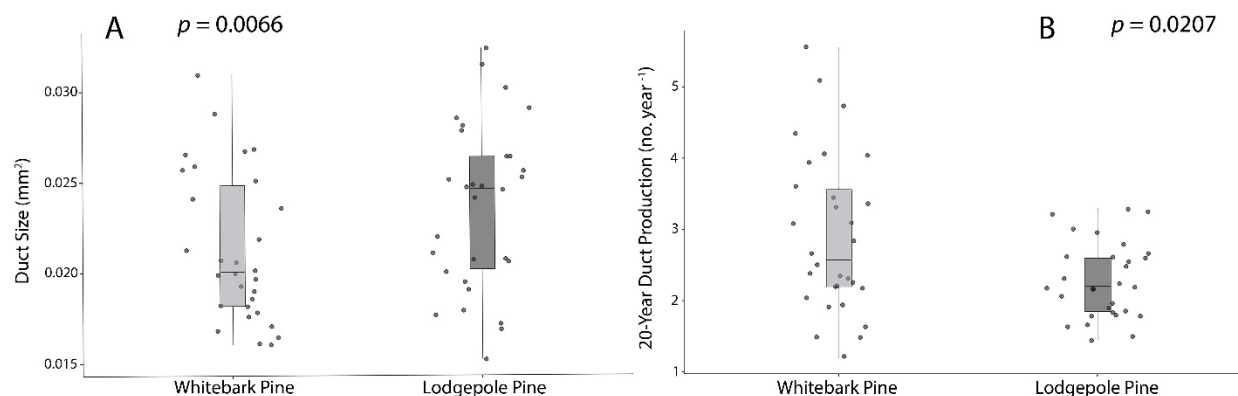
Figure 10. Comparison of resin duct production (A) and growth (basal area increment; B) across pairs of lodgepole and whitebark pine over the 20-year period prior to sampling (1998–2018).

Unstandardized resin duct metrics (size, area, and production) correlated positively with growth (BAI) for both species, while standardized duct metrics (duct density and relative duct area) correlated negatively with growth. Specifically, resin duct production was strongly, positively

correlated with growth in whitebark pine ($r = 0.59$, $p = 0.0007$) but not in lodgepole pine (**Table 5**). Resin duct size and growth were positively correlated in both whitebark pine ($r = 0.52$, $p = 0.0034$) and lodgepole pine ($r = 0.56$, $p = 0.0015$). Similarly, resin duct area and growth were strongly, positively correlated in whitebark ($r = 0.71$, $p < 0.0001$) and lodgepole ($r = 0.55$, $p = 0.0016$). In contrast, resin duct density and growth were negatively correlated in whitebark pine ($r = –0.36$, $p = 0.0539$) and lodgepole pine ($r = –0.55$, $p = 0.0019$; **Table 5**). Relative duct area was also negatively correlated with growth in whitebark ($r = –0.6$, $p = 0.0005$) and lodgepole pine ($r = –0.61$, $p = 0.0004$).

Tree size (DBH) positively correlated with growth (BAI) for both lodgepole ($r = 0.7$, $p < 0.0001$) and whitebark pine ($r = 0.77$, $p < 0.0001$; **Table 5**). In whitebark pine, tree size was also positively correlated with resin duct production ($r = 0.39$, $p = 0.0352$), duct size ($r = 0.46$, $p = 0.0098$), and duct area ($r = 0.56$, $p = 0.0014$). Tree size negatively correlated with resin duct density in whitebark pine ($r = –3.7$, $p = 0.0422$) and relative duct area in lodgepole ($r = –0.45$, $p = 0.01179$) and whitebark pine ($r = –0.58$, $p = 0.0008$; **Table 5**).

Table 5. Spearman correlation coefficients of tree ring growth and resin duct anatomical measurements in whitebark (*P. albicaulis*) and lodgepole pine (*P. contorta* var. *latifolia*). Correlations were conducted on metrics averaged over the full time series (1914–2018).

Pinus albicaulis	DBH	Growth (BAI)	Duct Production	Duct Size	Duct Area	Duct Density	Rel. Duct Area
Growth (BAI)	**0.77**						
Duct Production	**0.39**	**0.59**					
Duct Size	**0.46**	**0.52**	0.11				
Duct Area	**0.56**	**0.71**	**0.63**	**0.84**			
Duct Density	**–0.37**	**–0.36**	**0.36**	**–0.48**	–0.17		
Rel. Duct Area	**–0.58**	**–0.6**	0.01	–0.34	–0.24	**–0.85**	
Pinus contorta							
Growth (BAI)	**0.69**						
Duct Production	0.12	0.08					
Duct Size	0.25	**0.56**	–0.06				
Duct Area	0.24	**0.55**	**0.36**	**0.9**			
Duct Density	–0.35	**–0.55**	0.25	**–0.62**	**–0.45**		
Rel. Duct Area	**–0.45**	**–0.61**	0.14	–0.32	–0.22	**0.87**	

Note: Bold values indicate significant correlations ($p < 0.05$). DBH = diameter at breast height; BAI = basal area increment

Relationship of Overstory Competition and Resin Chemistry

Stand density index (SDI) was uncorrelated with tree size, tree growth, and resin duct metrics for both species (**Table 6**). SDI was also uncorrelated with constitutive mono- and sesqui- terpenes for lodgepole pine (**Table 6**). However, in whitebark pine there was a significant negative correlation between monoterpenes and SDI ($r = –0.38$ $p = 0.03597$; **Table 6**), indicating monoterpene concentrations declined with increasing stand density.

Lodgepole pine basal area comprised approximately 55% of near-tree competition, followed by whitebark pine (25%), subalpine fir (12%), Engelmann spruce (5%), and Douglas-fir (3%). Engelmann spruce was consistently in the top performing models predicting constitutive

monoterpenes as a function of near-tree competition (**Table 7**). In addition, constitutive mono- and sesqui- terpene concentrations in whitebark pine were negatively affected by near-tree competition with Engelmann spruce ($p = 0.0227$) while no relationships were found for lodgepole pine. Generally, constitutive levels of monoterpenes in whitebark pine decreased with increasing Engelmann spruce basal area.

Table 6. Spearman correlation coefficients of stand density (Reineke's Stand Density Index; SDI), tree size (DBH), tree ring growth (BAI), and resin duct anatomical measurements for whitebark pine (*P. albicaulis*) and lodgepole pine (*P. contorta* var. *latifolia*). Correlations were conducted on metrics averaged over the full time series (1914–2018).

	Stand Density Index (SDI)	
	Pinus albicaulis	*Pinus contorta*
Monoterpene concentration	**−0.38 ($p = 0.03597$)**	0.09
Sesquiterpene concentration	−0.29	−0.24
Monoterpene Diversity (*H'*)	−0.26	0.16
Sesquiterpene Diversity (*H'*)	−0.24	−0.34
DBH	−0.05	−0.09
Growth (BAI)	−0.05	−0.28
Duct Production	0.01	−0.05
Duct Size	−0.17	−0.33
Duct Area	−0.15	−0.33
Duct Density	−0.05	0.35
Relative Duct Area	−0.22	0.28

Note: Bold values indicate significant correlations ($p < 0.05$). DBH = diameter at breast height; BAI = basal area increment

Table 7. Generalized linear models predicting constitutive monoterpenes as a function of overstory competition. Log likelihood reflects the probability of the data given the model, while K represents the relative number of sample parameters for each model. Top five models are shown as well as intercept-only model for reference.

Model	AICc	ΔAIC	LogL	K
SPC + PIEN + PSME + PICO + [PIEN * SPC] + [PICO * SPC]	1159.6	0.00	−570.39	6
SPC + PIEN + PSME + [PIEN * SPC]	1160.7	1.12	−573.57	4
SPC + PIEN + [PIEN * SPC]	1161.3	1.69	−575.09	3
SPC + PIEN + PSME + PICO + [PIEN * SPC] + [PSME * SPC] + [PICO * SPC]	1161.3	1.75	−569.87	7
SPC + PIEN + PSME + PICO + ABLA + [PIEN * SPC] + [PICO * SPC]	1161.8	2.21	−570.10	7
Intercept-Only Model	1168.8	9.2	−582.28	1

Abbreviations: SPC (species), PIEN (Engelmann spruce competitors), PSME (Douglas-fir competitors), PICO (lodgepole pine competitors), ABLA (subalpine fir competitors).

Discussion

Live / Dead Whitebark Pine Tree Pairs

Consistent with previous research (Kane and Kolb 2010, Ferrenberg et al. 2014, Hood et al. 2015), we found that live and dead trees exhibited notably different growth and defense characteristics. Live whitebark pine produced larger resin ducts with a greater annual investment in resin-based defenses than the whitebark pine that died. Over the entire time series, resin duct

size, duct area, and relative duct area were greater in live whitebark pine (by 56%, 48%, and 57%, respectively). In contrast, whitebark pine that died exhibited 22% greater growth over the full time series than their live counterparts and also produced 20% more resin duct structures on average. The increased growth was pronounced early in the record (~1919–1975) and shifted notably after 1975, whereby growth declined sharply. While those trees that died produced a greater number of resin ducts on average and consistently had a higher density of resin ducts across all time periods, the relative area of resin ducts was lower than live trees at both sites (**Figures 4, 6**).

Resin duct size, duct area, and relative duct area were the most important variables influencing whitebark pine survivorship (**Figure 6**). Trees producing larger ducts with a greater overall percentage of annual carbon investment were far more likely to survive disturbance events at each of the study sites. The presence of larger resin ducts and greater overall resin duct area in live trees could be associated with an increased capacity to mobilize oleoresin in response to attack or infection and may be a factor in the ability of live trees to endure numerous disturbance events over time. Although dead trees produced more resin duct structures the ducts were smaller with less overall area, which might have been insufficient to produce, store, and mobilize adequate amounts of oleoresin in response to wounding by bark beetles and blister rust infection (Hood and Sala 2015). This reduced resin flow in dead trees could be linked to lowered defense and higher mortality despite increased density of ducts, particularly in the years leading up to death.

Importantly, our results suggest that whitebark pine trees that invest a relatively greater amount of resources into the production of constitutive resin-based defenses have a higher probability of surviving disturbance events. This parallels previous research on other conifers (Kane and Kolb 2010, Ferrenberg et al. 2014, Hood and Sala 2015, Hood et al. 2015, Hood et al. 2016, Zhao and Erbilgin 2019, Zhao et al. 2019).

Resin Chemistry, Growth, and Defense in Whitebark and Lodgepole Pine

Our 104-year record comparing growth and defense characteristics within and between whitebark pine and lodgepole pine yields four important findings. *First*, we did not find evidence of a tradeoff between tree growth and tree defenses (resin duct morphology and constitutive resin chemistry; **Tables 4, 5**). This suggests that trees growing under favorable field conditions can experience high growth rates and be well defended. We suspect that this likely reflects the highly variable site quality of natural forest stands rather than an absence of within-plant allocation posited by Herms and Mattson (1992). That is, within-plant allocation patterns do not necessarily scale up to between-plant inverse relationships (and may even be reversed) at the landscape scale (Howe et al. 2020). *Second*, we found that resin ducts and constitutive mono- and sesqui-terpenes are not related in lodgepole pine while duct size, production, and area were positively related to constitutive terpenes in whitebark pine (**Table 4**). We interpret the lack of clear relationships among tree growth, resin duct morphology and resin chemistry to be related to complex evolutionary processes shaping bark beetle-host interactions. While mountain pine beetles have evolved to exploit chemical signatures in the identification and colonization of individuals, trees present bark beetles with innumerable permutations of physical and chemical defenses. A beetle cannot readily predict resin duct architecture during host identification and colonization. Thus, trees with well-developed resin duct defenses have an increased advantage of

enduring bark beetle attack. This is supported by research demonstrating the importance of resin duct anatomy in surviving beetle activity for a variety of conifers (Kane and Kolb 2010, Ferrenberg et al. 2014, Hood and Sala 2015, Hood et al. 2016, Mason et al. 2019, Zhao and Erbilgin 2019, Zhao et al. 2019, Kichas et al. 2020). *Third*, based on constitutive terpene profiles (**Figures 8, 9**), bark beetles are more likely to enter lodgepole pine but more likely to successfully elicit mass attacks in whitebark pine, which agrees with beetle attack and success patterns in the field (Bentz et al. 2015). Specifically, whitebark pine produced greater quantities of compounds known to enhance beetle success, including (–)-α-pinene, which beetles utilize as precursor for the biosynthesis of their aggregation pheromone, (–)-*trans*-verbenol (Blomquist et al. 2010, Keeling 2016). Whitebark also produced more β-myrcene, which mountain pine beetles utilize as synergists for pheromone attraction (Borden et al. 2008). While lodgepole pine produced less of these compounds we found significantly greater levels of α-phellandrene and β-phellandrene, which mountain pine beetles exploit for host recognition. Lodgepole pine also produced more compounds that inhibit beetle fitness, including (+)-δ-limonene, which is highly toxic to mountain pine beetles, and 4-allylanisole (aka estragole), which inhibits attraction to pheromones (Hayes and Strom 1994). Thus, despite having fewer exploitable compounds for pheromone production and enhancement, the increased concentration of compounds important for host identification likely results in mountain pine beetles identifying lodgepole pines for colonization over co-occurring whitebark pine under endemic beetle activity, while under mass attack and outbreak scenarios, beetles are more likely to succeed when colonizing whitebark pine trees. *Fourth*, overstory competition, particularly by Engelmann spruce, can influence tree defenses, specifically constitutive terpene concentrations (**Tables 6, 7**). We found evidence to suggest that Engelmann spruce interacts with whitebark pine, resulting in decreased monoterpene concentrations with increasing Engelmann spruce presence. This trend was observed in whitebark pine despite Engelmann spruce occupying a small percentage of local competition. In addition, Engelmann spruce was a comparatively strong predictor of constitutive monoterpenes for whitebark pine compared to other competitors. Whether these effects are attributable to the microsites where Engelmann spruce resides or to more nuanced direct and indirect effects of Engelmann spruce ecology (e.g., changes in soil characteristics through litter deposition, root exudates, and unique microbial assemblages) remains unclear. This is an important point that should be further investigated. Competitive tree interactions could lead to altered bark beetle-conifer interactions as host and nonhost species migrate in response to changing climate.

Conclusions and Implications

Whitebark pine individuals within both of our sites appear to exhibit differing strategies in the allocation of resources toward growth and defense. When considering trees that died and residual live trees that persisted through 20th century disturbance events, live trees produced larger resin ducts with a greater overall annual duct area relative to growth. In contrast, those trees that died invested more into growth, at the expense of defense. Both strategies involve tradeoffs that can confer fitness benefits under different circumstances. As defensive features are energetically expensive to produce and maintain it is difficult to generalize what strategies may be most appropriate under what circumstances. This is an important point when considering contemporary breeding and planting programs, which have been identified as major restoration initiatives in many jurisdictions (Sniezko 2006, Keane et al. 2012). Within these programs seedlings are traditionally selected for based on growth-related characteristics with little

consideration for defensive physiology (White et al. 2007). Our results lend insight into resin duct characteristics that may be beneficial to increasing survivorship and promoting whitebark pine retention, which is particularly important given the sensitivity of whitebark pine to unforeseeable changes in disturbance regimes.

In regard to resin chemistry, we found differences in whitebark and lodgepole pine growth and physical / chemical defenses that have important implications for bark beetle behavior and forest management. These results have several important implications. From the perspective of a bark beetle, whitebark pine appear more chemically conducive for reproductive success while lodgepole pine are more attractant for primary colonization. Under endemic scenarios mountain pine beetle persist in low numbers, colonizing stressed individuals with depleted defense systems. However, during outbreak conditions, beetle populations can overwhelm defenses of vigorous, well-defended trees. As a result, even the healthiest whitebark pine growing within this mixed-conifer setting may be increasingly vulnerable to beetle-induced mortality. However, conifers have co-evolved with insects and have developed complex defensive strategies in response to these dynamic pressures. Resin duct architecture can facilitate oleoresin production and mobilization in ways that are not readily perceptible to beetles. The lack of clear relationships between these various defensive features highlights the intricate, but complex, nature of the biophysical relationships that connect them. In addition, local competition and species-specific interactions may further influence growth and defense characteristics, leading to unpredictable outcomes as species migrate in response to a warming climate.

Managers tasked with preserving and promoting whitebark pine populations are presented with increasingly limited options for achieving successful outcomes. Many management prescriptions rely on surficial performance indicators (primarily growth) that may fail to account for the important role of physical and chemical defenses in moderating disturbance interactions. Few studies have investigated patterns of growth and defense using multiple proxies (resin duct anatomy and resin chemistry) and there is a need to expand this research to a broader range of species and environments.

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Appendix A: Contact Information

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Appendix B: List of Deliverables

Publications in Peer-reviewed Journals		
Status	**Title**	**Journal**
Published 12/20	Whitebark Pine (*Pinus albicaulis*) Growth and Defense in Response to Mountain Pine Beetle Outbreaks	*Forest Ecology & Management*
In preparation	Growth and Defense Characteristics of Whitebark Pine (*Pinus albicaulis*) and Lodgepole Pine (*Pinus contorta var latifolia*) in a High Elevation, Disturbance Prone Mixed-Conifer Forest in Northwestern Montana, USA	TBD
In preparation	Climatic and Environmental Factors Influencing Whitebark Pine (*Pinus contorta*) Growth and Defense Across Two Geographically Distinct Populations in the Northern Rocky Mountains	TBD

Doctoral Dissertation		
Status	**Title**	**Academic Institution**
In progress Anticipated completion Summer 2021	Growth and Defense Characteristics of Whitebark Pine in Disturbance Prone Mixed-Conifer Forests of the Northern Rocky Mountains	Montana State University

Other Deliverables		
Type	**Status**	**Title**
Presentation *Whitebark Pine Ecosystem Foundation*	Completed (9/2019)	Whitebark Pine (*Pinus albicaulis*) Growth and Defense in Response to Mountain Pine Beetle Outbreaks
Presentation *Ecological Society of America*	Completed (8/2020)	Whitebark Pine (*Pinus albicaulis*) Growth and Defense in Response to Mountain Pine Beetle Outbreaks
Presentation *Research & Management of High Elevation Five Needle Pines*	Pending (8/2021)	Growth and Defense Characteristics of Whitebark Pine (*Pinus albicaulis*) and Lodgepole Pine (*Pinus contorta var latifolia*) in a High Elevation, Disturbance Prone Mixed-Conifer Forest in Northwestern Montana, USA
Presentation *Ecological Society of America*	Pending (9/2021)	Growth and Defense Characteristics of Whitebark Pine (*Pinus albicaulis*) and Lodgepole Pine (*Pinus contorta var latifolia*) in a High Elevation, Disturbance Prone Mixed-Conifer Forest in Northwestern Montana, USA
Publication *Nutcracker Notes Periodical*	Published (4/2020)	Whitebark Pine (*Pinus albicaulis*) Growth and Defense in Response to Mountain Pine Beetle Outbreaks
Field Tour *Whitebark Pine Ecosystem Foundation / CSKT*	Completed (9/2019)	Whitebark Pine as a Culturally Valuable Resource on the Flathead Indian Reservation
Webinar *Northern Rockies Fire Science Network*	Scheduled (2/2021)	Growth and Defense Characteristics of Whitebark Pine in Disturbance Prone High Elevation Montane Ecosystems

Appendix C: Metadata

Upon completion of the student's dissertation program (tentatively scheduled for Summer 2021), all growth and defense data associated with this project (.xlsx and .rwl files) will be submitted to the International Tree Ring Database following FGCD-STD guidelines.